Epibionts of the Australian Eastern Long-necked Turtle (*Chelodina longicollis* Shaw) from farm dams

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ABSTRACT

Ectoparasitic and ectocommensal macroinvertebrates (epibionts) of turtles have received limited attention with species from most Australian chelids poorly known. In this paper we present observations on the taxa collected from the widespread and abundant Australian freshwater species *Chelodina longicollis* Shaw, the Eastern Long-necked Turtle, sampled from farm dams in North Western peri-urban Sydney. Twelve epibiont taxa were collected, with the most commonly encountered species being the leech *Placobdelloides bancrofti* McKenna and a chironomid. Encounter rates varied over winter, and with carapacal algal mass, and farm dam. In contrast, they do not show a preference based on turtle sex. Since *C. longicollis* of different size and sex show habitat selectivity within farm dams (Ryan and Burgin 2007), epibiont distribution is not due merely to opportunism.

Key words: parasite, leech, habitat selection, invertebrate richness, aquatic turtles, invertebrate diversity, freshwater turtle, tortoise

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Introduction

Ectoparasitic and ectocommensal macroinvertebrates (epibionts) of turtles have received limited attention. However, within this context, marine species (Caine 1986; Choy *et al.* 1989; Frick and Slay 2000; Greenblatt *et al.* 2003; Kitsos *et al.* 2005) have been most studied. Publications on the species associated with freshwater turtles have been predominantly taxonomic/phylogenetic (Richardson 1967, 1968a, 1968b, 1969, 1970; Jennings 1985; Sluys 1990; McKenna *et al.* 2005) or range extensions (Tucker *et al.* 2005). Other data have been incorporated as minor contributions to theses (Parmenter 1976; Chessman 1978) and ecological studies have been largely restricted to leeches. There have been only rare examples of papers that cover the fauna more generally (Hulse 1976).

Placobdella leech species are the most widely encountered leeches of freshwater turtles (Table 1). While some leech species are parasitic (e.g. *Erpobdella punctata* Leidy), others (e.g. *Placobdella rugosa* Verrill) appear to be non-parasitic commensal species (Richardson 1969; Chessman 1978).

Parmenter (1976) commented that the parasites of Australian chelids (a sub-set of the Australian freshwater turtle taxa) were undescribed or, at best, their life cycle was poorly known. Few papers (Jennings 1985; Sluys 1990; McKenna *et al.* 2005; Tucker *et al.* 2005) have been published since that time. These have been largely associated with species description. The ecology of these epibionts therefore continues to be neglected and there are no published data that focus on the non-leech commensal fauna. In this paper we present observations on the parasitic and commensal epibionts of the widespread and abundant Australian freshwater turtle species, *Chelodina longicollis* Shaw.

Methods

The study was undertaken in farm dams in the Eastern Creek Catchment, approximately 40 km north-west of Sydney Central Business District (33°39'S - 33°51'S, 150°45'E - 150°53'E) between April and September 1995. Rainfall was unevenly spread over this period with much lower than average rainfall recorded each month from April to August compared to the long term average (Figure 1). The winter temperatures generally tracked the maximum monthly average although August was warmer than the average. There were 12 days over 25°C compared to the average historic daily maxima (18.9°C) for August in the study locality (Figure 2).

Four dams were sampled monthly from April to September 1995, and in the final month (early spring) 11 additional dams were sampled. Each month, two unbaited fyke nets were set at randomly determined positions at the edge (≤ 1.5 m depth) of dams with floats placed within the nets to ensure that the turtles were able to breathe at the surface while trapped in the nets (*cf.* Burgin *et al.* 1999). Netted turtles were collected the following morning and sexed (*cf.* Chessman 1978), the carapace length measured, and they were uniquely marked (*cf.* Cagle 1939).

Table 1. Previous recordings of leech, host species of turtle and reference.

Leech species	Turtle species	Reference
Bogabdella diversa	*Emydura macquarii*	Richardson 1969 Chessman 1978
Erpobdella punctata	*Kinosternon sonoriense LeConte*	Hulse 1976
Placobdella bancrofti	*E. macquarii* *Chelodina longicollis*	Richardson 1968a Parmenter 1976
P. ornate	*Chrysemys guttata Schneider*	Foster and Sawyer 1974
	Chry. picta Schneider	
	Chry. serpentine Linnaeus	Sawyer *et al.* 1986
	Graptemys pseudogeographica Kohnii	Sawyer *et al.* 1986
	Pseudemys scripta Schoepff	
	Sternotherus odoratus	Sawyer *et al.* 1986
	Latreille	Sawyer *et al.* 1986
P. parasitica	*Clemmys guttata Schneider*	Ernst 1976
	Chel. insculpta De Vis	Koffler *et al.* 1978
	Chry. picta Schneider	Ernst 1971
	Chrysemys picta belli Gray	MacCulloch 1981
	C. serpentine	Sawyer 1986
	G. pseudogeographica	Sawyer 1986
	P. scripta	Sawyer 1986
	S. odoratus	Sawyer 1986
P. rugosa	*K. sonoriense*	Hulse 1976
Placobdellid sp.	*C. longicollis*	Chessman 1978
	C. expansa	Chessman 1978
	E. macquarii	Chessman 1978
Richardsonianus australis Bosisto	*C. longicollis*	Chessman 1978

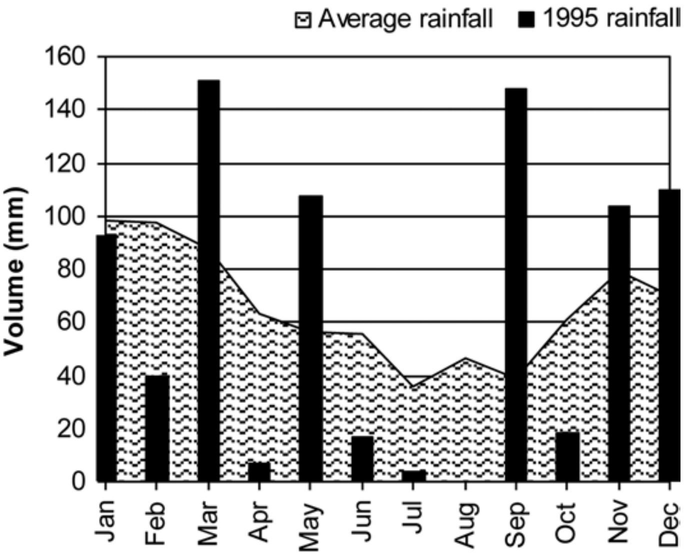

Figure 1. Western Sydney average monthly rainfall for 1995 compared to the 50 year average.

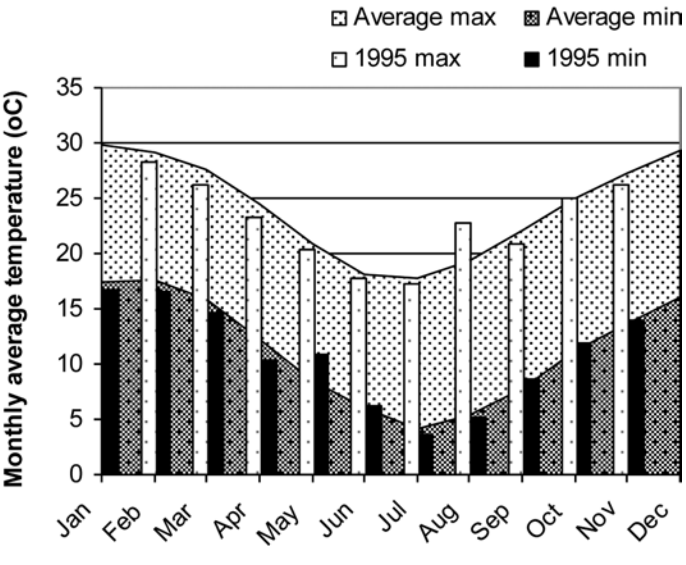

Figure 2. Western Sydney maximum and minimum monthly average temperature for 1995 compared to the 50 year average.

The density of algae on the carapace was recorded using a visual four-point scale. To initially develop the scale, turtles were placed in an aquarium with clear water to determine coverage and length of algae. Index 1 was designated as turtles with limited coverage, determined by the algae being insufficiently long to float above the carapace when the turtle was placed in the water-filled aquarium. Turtles were assigned to Index 2 if the algae were such that they floated above the carapace and coverage of the carapace was < 80%. Turtles assigned to Index 3 had algae that were at least 2.5 cm long and the carapace had effectively 100% algal coverage. Turtles were assigned to Index 4 when they had a dense growth of algae over the carapace, with growth elsewhere on the body, such that the turtle was effectively camouflaged due to the algal length, density and coverage.

Each turtle was examined for epibionts on the body, including the carapace, and within the algal mass, before release at point of capture. Epibionts that were removed were stored in 70% ethanol and returned to the laboratory for identification under a dissecting microscope (x 40 magnification).

A series of Chi-square test of association analyses were used to investigate the association with turtle sex, turtle size (after converting continuous to discrete interval data), and dam of capture, with epibionts from the turtles and its associated algal mass. The presence of epibionts on turtles from farm dams that were sampled over six months was also tested against month of capture, turtle size, and sex.

Results

Of the 158 *C. longicollis* sampled, 90.5% supported epizoic carapacal growth of the filamentous green algae *Basicladia ramulosa* Ducker. Most of these had an intermediate algal mass (34.2% Index 2, 38.6% Index 3), only 17.7% had a dense mass consistent with Index 4. The only four turtles caught in April or May, and then recaptured four or five months later showed an increase in algal growth that was consistent with a change of one to two visual indices. This indicated that the algae growth continued through winter. The only other recapture, a turtle caught in two successive months, showed no visual change in algal mass. The density of algal mass on the carapace was not statistically associated with sex, although it varied with turtle size ($\chi^2_{3,\,0.001}$ = 15.49), larger turtles carried a greater density of algae. A significant difference was found in the density of the algal mass on turtles among dams ($\chi^2_{9,\,0.001}$ = 25.24) although there was no identified pattern to the differences investigated.

Eleven species of epibiont were collected from 88 *C. longicollis*, and the turtles most commonly carried leeches (38%, n = 158). *Placobdellpoides bancrofti* McKenna were found primarily on the carapace, although they also occurred on the shell and skin near the tail and head, and on the plastron. A second, unidentified Hirudinea species was found in clusters attached to the skin, primarily in the tail area on 8.2% of turtles. Chironomid flies were also relatively commonly carried by turtles (31.6%) and were encountered primarily within the algal mass. Most other epibionts were found on few turtles. There were differences in the presence of epibionts on *C. longicollis* over the study period ($\chi^2_{3,\,0.0001}$ = 21.17, Table 2). There was an increase in the number of turtles with epibionts in the cooler months compared to warmer months of the study period. There was also a significant difference among dams in the percentage of turtles carrying epibionts ($\chi^2_{3,\,0.01}$ = 12.52, Table 2), although the reason was not identified. The presence of epibionts was significantly different due to density of algal mass ($\chi^2_{3,\,0.01}$ = 13.95). As the density of carapacal algae increased from Index 1 - 3, the chance of encountering epibionts increased. However, turtles with the densest algal mass (Index 4) were less likely to carry epibionts than those with substantial carapacal algae (Index 3, Table 3). The presence of epibionts was not significantly associated with sex or animal size.

Table 2. Total number of *Chelodina longicollis* carrying epibionts, together with their diversity, in farm dams (Dams A – D sampled April to September 1995, E – O sampled in September only) in the South Creek Catchment, Western Sydney.

Dam ID[1]	Number of turtles	Turtles with epibionts	Diversity of epibionts/dam
A	33	26	2
B	14	11	2
C	31	20	3
D	24	9	4
E	2	1	1
F	2	1	1
G	13	4	4
H	2	1	1
I	4	0	0
J	5	0	0
K	9	4	6
L	1	1	1
M	4	3	3
N	14	7	4
Total	**158**	**88**	

[1] No turtles were captured in farm dam O

Discussion

A total of 12 epibiont species was collected from 158 *C. longicollis* across the coolest period of the year (April to September). The observation that the number of turtles with epibionts increased as the season progressed is counter-institutive[1].

Hart (1985) reviewed factors that influenced the distribution and abundance of epibionts in inland waters across the Southern Hemisphere. He concluded that while seasonality was more marked in temperate latitudes than tropical clines, the amplitude differed with differences in temperature. He suggested that temporal differences in stream fauna was an adaptation to unpredictable seasonal cycles, which led to greater seasonal flexibility and opportunism compared to the northern hemisphere. With particular reference to small, shallow wetlands, which would characterise the farm dams where we sampled turtles, Hart (1985) commented that physiochemical profiles influence a range of fauna. Since in the year we sampled, temperatures typically tracked the long-term average (Figure 2), the 'unusual' increase in epibionts across winter could simply reflect the relatively mild temperatures compared to winters in some other temperate areas of Australia. The observation that carapacal algal mass increased over the study demonstrated that surface waters were also sufficiently warm for algal growth during the season.

McKenna *et al.* (2005) collected *P. bancrofti* from *Emydura krefftii* Gray and *Elseya latisternum* Gray in northern Australia, while in the inland south-east Murray River catchment, Chessman (1978) recorded that placobdellids were encountered on all species (*C. longicollis*, *Chelodina expansa* Gray, *Emydura macquarii* Gray), in all localities where 'intensive collection' occurred. He observed that *P. bancrofti* were present on 51.3% (n = 152) of *C. longicollis,* and was relatively common on *C. expansa*, but were uncommon on *E. macquarii*. In our study, a Hirudinoidea species (possibly *Bogabdella diversa* Richardson, although based on Richardson (1969) this would be a range extension and a new turtle host) was collected from relatively fewer individuals (12.2%, n = 158). Conversely, *B. diversa* was not found on the 178 *C. longicollis* Chessman (1978) examined, although it was common on *E. macquarii* sampled in the same area. These observations indicate that leeches show host selectivity. However, as with the local freshwater turtles (Legler 1978; Chessman 1988), the leeches may select specific aquatic habitat, and their selection of turtle may be opportunistic. Conversely, since different sized turtles apparently select different habitats (Ryan and Burgin 2007), this could equally provide an explanation for differences in epibionts due to turtle size.

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[1] *C. longicollis* do not leave the water to bask (Cann, 1998).

Epibiont presence was independent of turtle sex. Chessman (1988) also concluded that epibionts did not differentiate between the sexes (he did not investigate correlation with size). Since a difference in population profile (sex, size) among dams has been previously observed in *C. longicollis* (Dalem 1998; Ryan and Burgin 2007), epibionts may be opportunistic. However, the presence of epibionts may also be influenced by the physiochemical characteristics of the water body (see e.g., Brainwood *et al.* 2004).

We found up to 20 egg capsules of the planarian *Bdellasimilis barwicki* Richardson in an armpit of only two individuals within a single dam. No adults were found in this farm dam. In the Murray River catchment capsules of *B. barwicki* have been collected from *C. longicollis* in large number, estimated to be 100 in a single arm-pit. They were also observed to be present throughout the year (Chessman 1978). The species is apparently a symbiotic predator of insect larvae and oligochaetes, and may also be an opportunistic commensal that may inadvertently ingest the host's discarded food (Jennings 1985). Since Richardson (1968a) suggested that *B. barwicki* probably remained on its host for long periods, and Chessman (1978) encountered the species throughout the year, *B. barwicki* may be rare in farm dams of the East Coast, compared to inland waterways at the similar latitudes.

Of the epibionts encountered in largest numbers, the Chironomidae were collected predominantly in the carapacal algal mass. Chironomids have been reported to have parasitic/commensal relationships with many other species, frequently other aquatic insects (Tokeshi 1995). This appears to be the first reported case of freshwater turtles acting as host. Chironomids have consistently high species richness throughout the year in standing water at this latitude and altitude (Wright and Burgin 2010), although they are typically present in fewer numbers during the cooler months, than in warmer months (Zalom 1981, Dehoney and Gaud 1983; Wright and Burgin 2010). If the chironomid species collected are not obligate symbionts of the algae *B. ramulosa,* the increase in growth in algal mass during the winter would have provided additional habitat that attracted these epibionts. The increase in chironomids may, therefore, be due to them transferring from other habitat/s within the farm dam to the algal mass on turtles.

While oligochaetes were not encountered in the current study, as indicated above Chironomidae insect larvae were one of the more common taxa encountered (Table 3). Some epibionts collected from *C. longicollis* make up a small portion of their diet, at least in the Murray River (eg. 0.1% chironomid, 0.1% gastropods, Chessman 1978). *Chelodina longicollis* collected from a pair of coastal dune lakes had a higher proportion of epibionts in their diet (e.g., 15.1% chironomid, 99.8% Trichoptera, Georges *et al.* 1986). Species, such as chironomids and gastropods, tend to feed at the interface between the sediments and the standing water (Williams 1980), while *C. longicollis* also tend to inhabit the lowest strata in the wetland, and they are adapted to spend extended periods under water (Legler 1978). The turtles, together

Table 3. Epibionts observed on *Chelodina longicollis,* together with a qualitative assessment of algae mass (negligible present [1] – substantial mass [4]), collected from farm dams in north western Sydney between April and September (cooler months) 1995.

Taxon	1	2	3	4	Total
Chironomidae sp. 1	4	4	25	18	50
Chironomidae sp. 2			1	2	3
Gastropoda - Planorbidae sp.				2	2
Glossiphonidae *Placobdella bancrofti*	5	14	20	7	47
Glossiphonidae (possibly) *Bogabdella diversa*		3	8	2	13
Insecta *Diptera* sp. Pupae			1		1
Turbellaria *Bdellasimilis barwicki* (egg capsules)			2		2
Nematode sp.			1		1
Invertebrate 11			1		1
Invertebrate 12			1		1
Unidentified egg capsules		1			1
Total	**9**	**22**	**60**	**31**	**122**

with their algal mass, therefore present a convenient habitat for grazing epibionts that provides an alternative to the farm dam's substrate. However, the observation that effectively all species, commensal and parasitic, were most commonly encountered on turtles with a substantial algal mass (Index 3), but not in the presence of dense growth, indicated that there may be substrate selection occurring.

This research indicated that the ectocommensal and ectoparasitic (epibiont) taxa tended to use turtle substrate of specific density of algal mass, and based on size of turtle. In contrast, they do not show a preference based on turtle sex. Since C. *longicollis* of different sizes and sex show habitat selectivity within farm dams (Ryan and Burgin 2007), epibiont distribution is not due merely to opportunism.

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